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Enkephalin Effects on Learning and Memory

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Three experiments were conducted to assess the effects of a met-enkephalin analog [D-Ala^2]-methionine enkephalinamide (DAME) on learning and memory of monkeys. Experiment demonstrated that DAME impaired multiple discrimination reversal learning by capuchin monkeys. The DAME effect was not caused by DAME's effect on the monkeys' response to distracting or irrelevant stimuli nor was it caused by increasing production of systematic errors. Experiment 2 revealed DAME enhancement of two-choice spatial delayed response performance by squirrel monkeys at long but not short delays. However, in Experiment 3 low doses (100 micrograms/Kg) of DAME impaired delayed response performance on nine-choice spatial delayed response. DAME did not exert disproportionate effects on systematic as opposed to nonsystematic errors in either Experiment 2 or 3. DAME clearly has differing effec on different types of learning and memory problems in primates. These results strongly indicate that DAME would not be an effective agent for improving human performance.

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ENKEPHALIN EFFECTS ON LEARNING AND MEMORY

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ENKEPHALIN EFFECTS ON LEARNING AND MEMORY

INTRODUCTION

A landmark event in the history of behavioral pharmacology was the discovery in the early 1970s of opiate receptors in the mammalian brain (Goldstein, Lowney, & Pal, 1971; Pert & Snyder, 1973; Simon, Hiller, & Edelman, 1973; Terenius, 1973). Later research showed that opiate receptors occurred in all vertebrate brains, from hogfish to humans. Since opiate receptors are not likely to have evolved without concomitant development of naturally occurring brain opiates, a vigorous search was initiated to identify the putative endogenous opiates. Hughs, Smith, Kosterlitz, Fothergill, Morgan, and Morris (1975) and Terenious and Wahlstrom (1974) soon reported opioid activity in extracts prepared from animal brains. Later research demonstrated that two pentapeptides, met- and leu-enkephalin, accounted for most of the opioid activity of the brain extracts.

Met-enkephalin and leu-enkephalin occur in different populations of neurons within the central nervous system (Larrson, Childers, & Snyder, 1979). In addition, met-enkephalin has a relatively high selectivity for mu receptors while leu-enkephalin has a high selectivity for delta receptors (Goodman, Fricker, & Snyder, 1983). Lewis, Mishkin, Bragin, Brown, Pert, and Pert (1981) reported that mu receptors are progressively denser and exert greater influence at higher levels of sensory complexity and integration in the cerebral cortex including the frontal areas. Therefore, the mu receptors may have a central role in mediation of selective attention. Furthermore, because of abundant reciprocal connections between these cortical areas and the amygdala, these authors suggested that the mu receptors may play an especially important role in mediating emotion produced selective attention. If this hypothesis is correct, administration of mu selective opioids should affect performance on learning tasks with emotion producing distractors because the opioids would alter attention towards the distractors.

Following identification and synthesis of met- and leu-enkephalin in addition to numerous related peptides and analogues, a large research effort was directed towards understanding the physiological and behavioral effects of these compounds. As expected, most, but not all, of the enkephalin related peptides had analgesic effects. However, they also produced a variety of behavioral effects when injected in microgram quantities either centrally or peripherally. The strong behavioral efffects following peripheral injection are consistent with the current opinion that opioid peptides are moderately capable of penetrating the blood brain barrier (Kastin, Nissen, Schally & Coy, 1976; Kastin, Olson, Schally, & Coy, 1979; Rapaport, Klee, Pettigre, & Ohno 1980). However, this should not be taken to imply that all behavioral effects following peripheral opioid peptide administration are caused

by the peptides' effects on central receptors as leu-enkephalin and met-enkephalin have been identified in areas outside the central nervous system (Hughes, Kosterlitz, & Smith, 1977). Behavioral effects of naturally occurring endogenous opioids include changes in activity level, food and water intake, grooming, vocalization, and even human clinical phenomena such as depression and schizophrenia (Olson, Olson, Kastin & Coy, 1981). However, the effects of enkephalin on learning and memory will be the focus of the experiments to be reported here.

Published data describing effects of opioid peptides on learning and memory have often been ambiguous. Specific testing procedures, timing of injections, route of administration, dosages, species of subjects, and several organismic variables can modify effects of opioid peptides and their antagonists (for a review see Olson, Olson, & Kastin, 1984, 1985). The ambiguity of reported data has resulted in varied interpretations of opioid effects on learning and memory. These interpretations range from the proposition that opioids affect learning and memory by altering the quality of reinforcers (Goeders, Ingham, Lane, & Smith, 1983) to a recent proposal that abnormally low levels of endogenous opioids are responsible for the memory deficits seen in Alzheimer's disease (Jolkkonen, Soininen & Riekkinen, 1984).

Most published research describing effects of opioid peptides on learning and memory has involved the use of aversive stimuli (usually electric shock). Results have been confusing and often conflicting; findings of both enhancement and interference have been reported on both learning and memory caused by both opioid peptides and their antagonists. For example, leu-enkephalin impaired and met-enkephalin enhanced learning and later retention of a Y-maze shock escape problem based on spatial discrimination in mice. Neither drug affected open field activity (Martinez, Olson, & Hilston, 1984). A critical problem that arises when aversive stimuli are used in learning and memory research is that opioid peptides may reduce the perceived aversiveness of these stimuli. The reduced aversiveness may, in turn, reduce the learned fear evoked by conditioned stimuli paired with the aversive stimuli. To further complicate the problem, aversive stimuli themselves evoke increased levels of endogenous opioids (see Bolles & Fanselow, 1982). It is therefore virtually impossible to determine an unconfounded effect of opioid peptides on learning of aversively motivated tasks. However, one important finding with potentially important theoretical consequences is emerging from the literature on aversively motivated tasks. Opioid peptides generally facilitate performance on tasks requiring behavioral inhibition, such as passive avoidance. They impair performance on tasks requiring active responses, such as active avoidance (see Olson et al., 1981). The effect of enkephalins on avoidance learning may be mediated through the pituitary-adrenal axis since removal of the adrenal medulla eliminates the effect of met-enkephalin and increases the

minimal effective dose of leu-enkephalin (Martinez & Rigter, 1982).

In paradigms involving conditioned autonomic responses, a potent met-enkephalin analogue, (D-ala^2)-met-enkephalinamide (DAME), attenuates acquisition of conditioned heart rate responding in the rabbit (Gallageher, Kapp, & Pascoe, 1982) and interferes with previously learned thermoregulatory responses in the cat (Clark & Bernardini, 1982). However, to claim opioid interference with learning and memory of autonomically conditioned responses on the basis of results of this type is unjustified because of the direct physiological effects of the administered opioid analogs. For example, administration of DAME decreases systolic blood pressure and heart rate and exerts a time dependent biphasic effect on colonic temperature in cynomologus monkeys (Owen, Gisolfi, Reynolds, & Gurll, 1984). DAME also induces feeding in the rat (Mclean & Hoebel, 1982), reduces motor activity in mice (Kamegama & Ukai, 1983) and produces a dose dependent increase in the frequency and amplitude of intestinal contractions in the dog (Burks, Hirning, Galligan & Davis, 1982). The widespread physiological changes produced by opioids pose obvious difficulties in determining whether DAME's effect on autonomically conditioned responses are a result of interference with learning, memory, motivation, or physical ability.

Few data have been published to date on the effects of opioid peptides on appetitively motivated tasks. No tests have been conducted on the hypothesis that opioids selectively binding to mu receptors will have particularly potent effects on learning problems incorporating an emotion producing distractor. The first report of learning effects from peripherally injected opioid peptides was published by Kastin, Scallon, King, Scally, and Coy (1976). These investigators demonstrated that met-enkephalin and [D-ala^2]-met-enkephalin increased running speed and decreased errors by rats learning a 12-choice Warden maze. In addition, an analogue, [D-phe^4-]-met-enkephalin that exerts negligible opiate effects also improved performance. This result indicated that the learning enhancement of the enkephalin was independent of the classical opiate effects. Kastin, Kostrzewea, Schally, and Coy (1980) demonstrated that rats treated with met-enkephalin as neonates displayed enhanced ability to learn a maze for food reward three months later. The enkephalin apparently increased the later learning abili', of the maturing rats. Olson, Olson, Kastin, Green, Roig-Smith, Hill, and Coy (1979) reported that a pentafluorinated met-enkephalin analogue facilitated performance of rhesus monkeys on the reversal, but not on the prereversal phases of multiple discrimination reversal problems. However, the same drug had no significant effect on 0, 30, and 60 sec spatial delayed response problems. A later study on delayed response with a different enkephalin analogue, [D-Phe^4]-met enkephalin showed an amnesic effect of the opioid peptide (Olson, Roig-Smith, Mauk, LaHoste, Coy, Hill, & Olson, 1981). Clearly,

the effects of ehkephalins on primate short term memory are still poorly understood. Linden and Martinez (in press) showed that leu-enkephalin at 300 micrograms/kg reduced memory by mice on a Y-maze spatial discrimination for food reward. However, lower (100 micrograms/kg) or higher (600 micrograms/kg) doses had no effect.

This report describes three experiments on the effects of DAME on appetitively motivated learning and memory problems in monkeys. The first is a direct test of the previously described hypothesis that opioids with mu receptor binding properties will exert particularly strong effects on problems with a strong emotional component. Mu receptors are densely distributed in the frontal cortex (Lewis et al., 1981) and the integrity of the frontal cortex is especially important for normal delayed response performance in monkeys (Pribram, 1973), DAME may accordingly have a significant effect on delayed response performances, a possibility examined in Experiments 2 and 3.

EXPERIMENT 1

The first experiment was designed to demonstrate the effects of DAME on learning of multiple discrimination reversal problems by capuchin monkeys. In order to perform successfully on the reversal phases of the problems, the monkeys had to inhibit acquired preferences to the stimulus rewarded during the prereversal phases. A previous experiment by Olson et al. (1979) indicated that DAME facilitated performance on reversal but not prereversal phases of multiple discrimination reversal learning by rhesus monkeys.

To test the hypothesis that DAME increases responsiveness to emotionally distracting stimuli, two sources of distracting stimulation were included as variables. The first source of potential distraction was irrelevant cues on the bases holding the discriminative stimuli. Capuchin monkeys display strong preferences and aversions to small multidimensional stimuli. Furthermore, these preferences and aversions persist after extended periods of testing with those stimuli (King & Fobes, 1974). The second source of distracting stimulation was recorded sounds from the capuchin monkey colony room.

Virtually all published reports on learning and memory in animals have used percentage of correct responses or some closely related measure as the only measure of performance. This approach does not allow for the possible importance of response sequence selection during learning. A well known truism about animal learning is that response sequences contain a mixture of random or unsystematic and nonrandom or systematic error producing response sequences (Harlow, 1959; Levine, 1965). The conventional approach to animal learning, which has been referred to as the uniformity hypothesis, is based on the implicit assumption that systematic and unsystematic errors undergo equal proportional changes as the overall percentage of errors changes. However, several examples of the failure of this assumption have been reported in animal learning (King & Fobes, 1974) and delayed response (Lentz & King, 1981). As DAME could express different effects on systematic and unsystematic errors, a procedure that we have referred to as Sequential State Theory (SST) was therefore used to partition responses into proportions accounted for by correct responding as well as random and various types of nonrandom error producing tendencies.

Method

Subjects

Subjects were five male capuchin monkeys (*Cebus apella*). All subjects had extensive prior experience on a wide variety of learning problems including discrimination learning-set, sameness-difference learning-set, and oddity learning-set.

Apparatus

The monkeys were tested in 32 by 35 by 41 cm stainless steel cages that were also used for transportation the monkeys between the animal colony and the testing room. The testing cage was placed in a modified Wisconsin General Testing Apparatus (WGTA). The stimulus objects were placed on a 35 by 15 cm stimulus presentation tray that contained two recessed foodwells located 18 cm apart center to center. The tray was mounted on two tracks that allowed the tester to move the tray towards or away from the monkey during the test trials. A pully operated opaque screen was interposed between the test cage during intertrial intervals. The tester viewed the stimulus presentation tray and the subject through a one-way-screen.

The stimuli were composed of two parts: discrimination objects and bases. A set of 192 discrimination objects was constructed from a wide variety of junk and hardware items differing in multiple dimensions and mounted on heterogeneously colored blocks measuring 4.4 by 4.4 by 0.64 cm. The bases were 25 masonite squares measuring 9 by 9 cm covered with distinctive and colorful pieces of wallpaper and contact paper. Attached to the bases were various objects including artificial worms and insects, brightly colored sequins, and pieces of cloth. The set of bases also included a pair covered with a homogenous light brown contact paper and no attached materials. The stimulus objects could be easily applied and removed from the bases by means of small strips of attached Velcro.

Procedure

Fifteen minutes before each test session, the monkeys were administered subcutaneous injections of physiological saline or [D-ala^2] methionine enkephalinamide (DAME) at dosages of either 100 or 800 micrograms per kg. Solutions were acidified with acetic acid to a ph of approximately 4.2.

The following procedure was used in presenting each trial of the multiple discrimination reversal problems. While the opaque screen of the WGTA was down, the tester baited one of the two foodwells with a small piece of raisin or marshmallow. Both foodwells were then covered with a base and an attached discrimination object. Each base fitted between two tracks on either side of the foodwell that prevented the monkey from capturing the object. The tester then raised the opaque screen and pushed the tray forward slowly. When the tray reached the front of the monkey's test cage, the tester depressed a footswitch to begin timing the monkey's response latency. The latency interval was terminated when the monkey responded by pushing back one of the discrimination objects, thereby exposing one of the foodwells. If the correct object was displaced, the monkey retreived the food from the foodwell. After the response, the tester lowered the opaque screen, withdrew the

screen, and prepared the tray for the next trial.

Experiment . Design

The monkeys were presented with two discrimination reversal problems during each test session of 54 trials. One problem had 15 prereversal trials and the other had 11. Both problems included 14 postreversal trials.

On half of the test days, recorded sounds from the capuchin monkey colony room were played. The recordings were made shortly after the monkeys had been highly aroused by the presence of a large dog in the colony room. Four different colony recordings were played in counterbalanced order over different days. On the other half of the test days, white noise was present during testing. The mean intensity of the colony sounds and the white noise was adjusted to approximately 70 db.

On each problem, two discrimination objects were simultaneously presented on each trial, one correct on prereversal trials, the other correct on postreversal trials. Each pair of discrimination objects was used on only one problem. During half of the test days, the two discrimination objects were placed on two different bases that were sources of irrevevant cues. The two bases for each problem were randomly selected from the set of 25 bases. No particular pair of bases was ever presented on more than one problem. Before the start of each problem with irrelevant bases, the monkey was allowed to choose between the two bases without attached objects. Either choice was rewarded. The selected base, designated as PB, was defined as the preferred one and the nonselected base, designated as NPB, was defined as the nonpreferred one. Response frequencies to the PBs were used in the subsequent SST analysis of systematic cues related to base preferences. The correct object was attached to one base on half of the trials of a problem and attached to the other base on the other half of the trials. Thus, any propensity of a monkey to approach or to avoid one of the objects would have resulted in a chance probability of reward. On the other half of the test days, both discrimination objects were mounted on the two identical bases covered with light brown contact paper.

The three drug conditions (saline, 100, and 800 microgram/kg DAME), two environmental sound conditions (colony sound or white noise), and two base cue conditions (irrelevant or constant base cues) yielded 12 unique combinations of independent variables. Each combination was presented once every 12 test days with the restriction that each drug condition was presented once every three days and no drug condition occurred on two successive days. Four 12 day blocks were presented for a total of 48 test days.

Sequences of rewarded positions on the tray (right or left) and, for irrelevant cue base problems, sequences of bases (PB or

NPB) that were positive were randomized with the following set of restrictions.

1. Right and left sides were correct on an equal number of trials.

2. All four possible two-trial sequences of correct side (viz. R-R, R-L, L-L, and L-R) were presented equally often.

3. A and B bases were correct on an equal number of trials.

4. All four possible two-trial sequences of rewarded bases (viz. PB-PB, PB-NPB, NPB-NPB, and NPB-PB) were presented equally often.

5. The four possible combinations of right or left side correct and PB or NPB base correct were each presented on an equal number of trials.

This extensive balancing was necessary in order to obtain unbiased estimates of the strengths of systematic errors related to position and base cues.

Response Sequence Analysis

The SST analysis begins by defining a list of possible constraints that could result in each systematic pattern of responses. One restraint is learning which would result in above chance choice of the correct stimulus. All other constraints are by definition error producing. Estimated strengths of these constraints as well as the strength of random or nonsystematic responding are obtained by calculating unbiased proportions of trials whose outcomes are consistent with each constraint. Simple linear transformations are then applied so that the summed strengths of the constraints is one.

SST estimates were calculated as if the monkeys were in one state on each trial. Each state has an associated type of response that always occurs when the subject is in that state. If the subject is in any other state, the response occurs with probability 0.5. Therefore, if A is the estimated probability of state A and Po(a) is the observed proportion of its associated response a, $A = 2Po(a) - 1$. The SST procedure is then simply a matter of defining a set of states that incorporate the most important constaints on responding and using the observed data to estimate Po(a) for each state. Po(a) is then used to obtain A, the estimated state strength and the dependent variable used in subsequent statistical tests. Data for irrelevant base problems were analysed over all two-trial sequences. These were catagorized into four 4 by 4 matrices based on whether responses (1) were correct (+) or incorrect (-), (2) were directed to the PB or to the NPB base, and (3) were to the right (r) or to the left (l) side. For problems without the irrelevant base cue, the PB and NPB base responses were omitted, leaving a single 4 by 4 matrix. Table 1 shows the response sequence matrices that were used.

a. State D (detect) reflects accurate remembrance of the correct stimulus and is associated with a correct (+) response. Therefore, $D = 2Po(+_2) - 1$, where $Po(+_2)$ was the observed proportion of sequences with a correct response on trial-2. In

Table 1

Response Sequence Matrix for Calculating State Strengths

	R_1R_2				R_1L_2			
	P_1P_2	P_1N_2	N_1N_2	N_1P_2	P_1P_2	P_1N_2	N_1N_2	N_1P_2
$+_1+_2$								
$+_1-_2$								
$-_1+_2$								
$-_1-_2$								

	P_1P_2	P_1N_2	N_1N_2	N_1P_2	P_1P_2	P_1N_2	N_1N_2	N_1P_2
$+_1+_2$								
$+_1-_2$								
$-_1+_2$								
$-_1-_2$								

Note: R and L indicate right or left response. P and N indicate preferred or nonpreferred base. + and - indicate correct or incorrect response. Subscripts 1 or 2 indicate first or second trial of successive two-trial sequences.

other words, $Po(+_2)$ was the summed frequencies across rows having a positive trial-2 outcome divided by the total number of sequences.

b. A potential source of errors in discrimination problems is a preexisting tendency to approach or to avoid the discriminative stimuli. If the preferred stimulus is positive, consecutive correct responses will occur; if the preferred stimulus is negative, consecutive incorrect responses will occur. This state, designated as S, has been referred to as object preference (see Harlow, 1959; Levine, 1965) and was associated with either two consecutive correct responses or two consecutive incorrect responses. The observed proportion of object preference responses, Po(s), was defined as the unweighted mean of the proportions of correct and incorrect two-trial sequences. Specifically, $Po(s) = [Po(+_2|+_1) + Po(-_2|-_1)] / 2$ and $S = 2Po(s) - 1$.

c. Another systematic source of errors is the tendency to base the current trial's choice on the position that was correct on the immediately preceding trial. This tendency could be manifested by tendency to choose the position that was correct on the preceding trial (positional win-stay;lose-shift) or by the opposite tendency to choose the position that was incorrect on the previous trial (positional win-shift;lose-stay). The former tendency was a manifestation of state F and the latter was a manifestation of state G. The observed proportion of win-stay;lose-shift responses has one component consisting of perseverative or stay responses following a correct response (St_2) and another component consisting of alternation or shift responses following an incorrect response (Sh_2). If these two estimates are weighted equally, the estimated proportion of positional win-stay;lose-shift responses $Po(w\text{-}s;l\text{-}s) = [Po(St_2|+_1) + Po(Sh_2|-_1)] / 2$ and $F = 2Po(w\text{-}s;l\text{-}s) - 1$. If positional win-shift;lose-stay responses predominates, F will be negative and $G = |F|$.

d. The most commonly reported systematic error in animal learning and memory experimentation is the simple position habit manifested by significantly more choices to one position than to the other. The state associated with position habit is B. If more right than left responses occurred on trial-two of the two trial sequences, the proportion of position habit responses $Po(b) = Po(r_2)$; $Po(l_2)$ was used if left responses predominated. It then follows that $B = 2Po(b) - 1$.

e. A second type of position based bias occurs if a monkey has an above chance tendency to choose the position selected on the previous trial, irrespective of whether that choice was correct or incorrect. The corresponding state was referred to as position perseveration, P, and the associated response was a perseverated right response r_2r_1 or a perseverated left response l_2l_1. The observed proportion of perseverative responses, Po(p), was then defined as the unweighted mean of the conditional proportions of right and left perseverative responses. Thus, $Po(p) = [Po(r_2|r_1) + Po(l_2|l_1)] / 2$ and $P = 2Po(p) - 1$.

f. On problems with an irrelevant base cue, a base

preference bias is possible which is analogous to position preference. Base preference, H, was manifested by an overall excess of responses to the preferred base PB. Therefore, Po(h) = Po(PB_2) where PB_2 was a response to the PB base on trial-two of the two-trial sequence and H = 2Po(h) - 1.

g. A second type of bias that could occur in irrelevant base cue problems is analogous to position perseveration. This state was base perseveration, I, which was manifested by two consecutive responses to the same base. Thus, Po(i) = [Po(PB_2 | PB_1) + Po(NPB_2|NPB_1)] / 2 where PB and NPB refer to choices of base PB or base NPB. It then follows that I = 2Po(i) - 1.

h. Some errors will be unsystematic to the extent that they are uncorrelated with stimuli, positions or outcomes on the current or the previous trials. This unsystematic responding was associated with the random state R. When state R was operative, both responses on a trial were assumed to be equally likely and therefore independent of any constraints that were associated with the other states. State R was defined as the proportion of responses remaining after responses attributable to all other states were subtracted. Therefore, if the other states have accounted for all significant systematic or nonrandom responses, R = 1 - D - S - F - B - P - H - I.

i. Finally, performance was expressed in terms of a statistic K that reflected correct responding independently of the influence of all systematic errors, namely those attributable to states S, F, B, P, H, and I. K was defined as D / (D + R), an adjusted value of D calculated as if the response sequence population consisted of only those sequences not attributable to systematic error. Therefore, K may be regarded as a bias free performance measure that has some conceptual similarity to bias free sensitivity measures used in signal detection theory (Green & Swets, 1974). One difference is that the SST approach allowed separation of sensitivity (or learning) from both sequentially dependent biases (viz. S, F, P, and I) and sequentially independent biases (viz. B and H). Signal detection theory allows separation of sensitivity from only one source of sequentially independent bias, an unrealistic restriction for animal learning and memory data.

Results

Since detect (D) was simply a linear transformation of the percentage of correct responses, statistical tests for both measures were identical. Percentage of correct responses decreased significantly with DAME [$F(2,8) = 5.15$, $p = 0.0356$]. However the 100 and 800 microgram doses yielded almost identical performances. In addition, the monkeys committed more errors on problems presented with irrelevant base cues than on problems presented with identical bases [$F(1,4) = 18.51$, $p = 0.0126$]. The monkeys also committed more errors on days when the recorded sounds from the capuchin monkey were played than on days when equally intense white noise was played [$F(1,4) = 1.87$, $p = 0.0122$]. Percentage of correct responses was low during

reversal than during prereversal trials [F(1,4) = 13.73, p = 0.0207]. No interactions were significant. Figure 1 depicts the effects of the irrelevant base cue and the monkey colony sounds over different dosages.

Analysis of the bias free measure K again showed a significant decline with DAME [F(2,8) = 4.89, p = 0.0411]. As with percentage of correct responses, the difference in K values for 100 and 800 microgram doses was negligible. K values were lower on reversal than on prereversal trials [F(1,4) = 12.75, p = 0.0233]. However, main effects involving base cues and type of ambient noise were no longer significant. The only significant interaction was between reversal and drug in [F(2,8) = 4.89, p = 0.0411] which was attributable to relatively low performance during reversal under all drug conditions.

Analysis of all systematic and unsystematic error sources showed no significant main effects nor any significant interactions involving the drug variable. In fact, none of the systematic error producing states had mean values differing significantly from zero. The mean value of random responding, R, was 0.736, a value differing significantly from zero [t(4) = 5.46, $p < .01$).

The mean response latency was 2.26 sec. Latencies did not vary significantly with level of DAME or with any of the other main variables.

Discussion

The principal result of this experiment was the small but significant increase in errors on multiple discrimination reversal problems caused by subcutaneous injections of DAME at dosages of 100 and 800 micrograms/kg. Dame did not, however, affect the mean response latencies. Therefore, the drug effect was probably not a consequence of changes in the monkeys' overall activity level or alertness. We had hypothesized that the drug effect would interact with two types of distracting stimuli: the irrelevant base cue and the recorded monkey colony sounds. The predicted interactions did not occur, although the irrelevant bases and the recorded colony sounds both significantly increased the error rate.

The DAME related performance decrement contrasts with the results of Olson et al. (1979) who reported that a fluorinated version of DAME improved performance on the reversal phase of multiple discrimination reversal problems. The different DAME effects could have been a result of species differences in the two experiments. Another possibly important difference in the two experiments is that Olson et al. (1979) used relatively inexperienced juvenile monkeys whereas we used highly experienced mature adults. Yet another possibility is that the extremely distinctive and colorful junk objects used in the present study elicited emotionally based preferences and

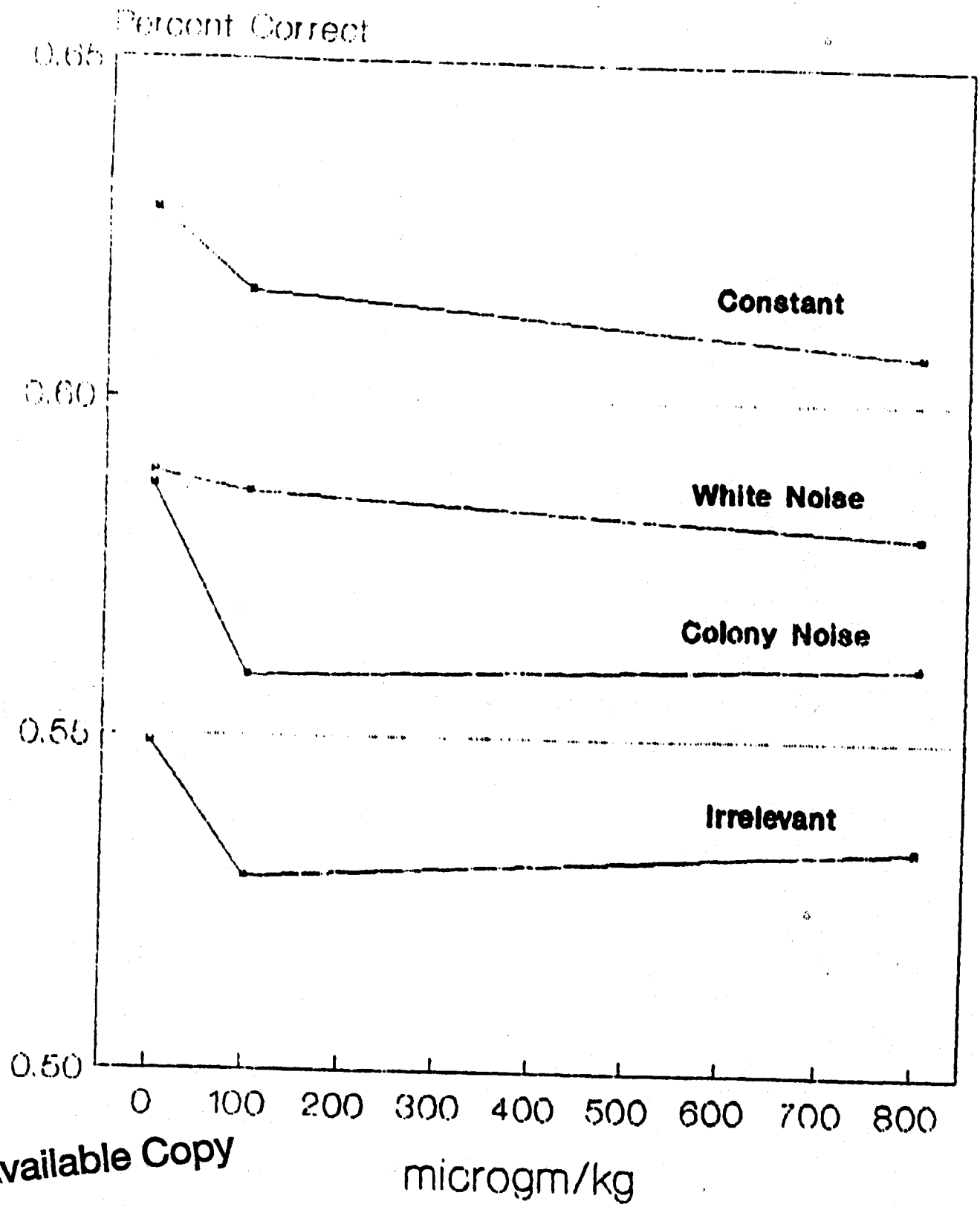

Fig. 1. Effects of distracting sounds and irrelevant base cues on percentage of correct responses in Experiment 1. Constant and Irrelevant refer to base cue conditions. Colony noise refers to recorded monkey colony sounds. Dosages of injected DAME are indicated along the abscissa.

aversions that were exacerbated by DAME. However, since object preference errors were not significantly greater than chance, this possibility is not compelling.

The capuchin monkeys' extensive prior experience with a wide variety of learning problems may have been responsible for the low frequency of systematic errors that consequently afforded no opportunity to test the effect of DAME on systematic errors. Strengths of systematic error producing states were simply too small to allow expression of drug effects. Thus, if DAME facilitates suppression of dominant but incorrect systematic errors, the effect would not have been manifested. As the juvenile rhesus monkeys tested by Olson et al. (1979) had received no prior training, they probably displayed a high level of systematic errors that could have been suppressed by DAME. This argument implies a biphasic effect of DAME in which it facilitates learning when strong sources of systematic errors are present but impairs learning when no strong sources of systematic errors are present. Unambiguous evidence for this hypothesis awaits an experiment demonstrating that DAME's effect varies with the relative proportion of errors that are systematic.

Finally, it should be noted that the lack of significant systematic errors associated with the base cues was not inconsistent with the significant increase in errors when the irrelevant base cues were present. This result indicates that the irrelevant base cue increased errors not by eliciting perseverative base preferences or aversions but by causing an overall increase in random responding. The significant decrease in the bias free measure K when irrelevant base cues were present supports this interpretation.

EXPERIMENT 2

The delayed response problem has a long history in behavioral testing of nonhuman primates (Fletcher, 1965; Jarrard & Moise, 1971; Medin & Davis, 1974). Delayed response and related problems involving short term memory have been widely used since they are often sensitive to organismic variables such as aging, radiation and drug effects. However, peptide effects on short term memory in appetitively motivated problems have been infrequently investigated. One study failed to demonstrate any significant effect of DAME on delayed response of rhesus monkeys (Olson et al., 1979) while another showed that a related met-enkephalin analogue impaired performance (Olson et al., 1981). However, both studies incorporated a WGTA and a direct delayed response procedure in which the tester signified the positive response locus by conspicuously baiting a stimulus tray foodwell and then placing an object over it. Performance was dependent upon the monkey's attention to the hand movements of the tester. Variability in the testers' food placement as well as emotional responses of the monkey to the presence of the tester's hand are possible sources of error with the direct procedure.

The indirect delayed response procedure affords greater precision and constancy in the predelay stimulus presentation than does the direct procedure. In the indirect procedure, the subject is trained initially to choose a response locus illuminated by a light. The presentation of the light then becomes the predelay cue (see Weiskrantz, 1968). Experiment 2 was designed to demonstrate the effects of DAME on indirect delayed response by squirrel monkeys.

Method

Subjects

Subjects were four male and two female squirrel monkeys (Saimiri sciureus) who were laboratory born with ages between four and five years. All monkeys had previously served in a series of experiments on delayed response with the apparatus used in the present study.

Apparatus

Monkeys were tested in the same stainless steel transport/testing cage used in Experiment 1. Removal of the guillotine door on one end gave the monkey access to a response panal containing two 5 cm square windows constructed from one-way-screen and recessed 2 cm from the panal's front surface. The two windows were located half way up from the floor, one on the right side of the panal, the other on the left side. Incandescent lights behind the two windows could illuminate small empty chambers thereby making them visable to the subject. The recessed walls of the two windows contained photocell units

that projected horizontal infrared beams in front of the windows. Interruption of either beam by the subject caused a response to be recorded. Correct responses were immediately followed by a 1 sec tone; incorrect responses simply activated a monitor light visable to the tester. A wooden block with a recessed foodwell on top was located below each window and held in place by magnets. The front surface of the block was flush with the front of the response panal. A transparent Plexiglas screen mounted in front of the response panal could be manually raised and lowered by the tester.

Procedure

Fifteen minutes before the start of each delayed response session, the monkey was administered a subcutaneous injection of only physiological saline or saline plus DAME at dosages of either 100 or 500 micrograms per kg. As in Experiment 1, the solutions were acidified to a ph of approximately 4.2.

At the start of each delayed response trial, one of the two window lights was illuminated for 2 sec. After a predetermined delay interval, the tester raised the Plexiglas screen. If the monkey interrupted the photocell beam in front of the previously lighted (correct) window, the 1 sec tone sounded immediately and the tester pushed the corresponding reward block toward the monkey giving it access to the reward. As soon as the monkey retreived the reward, the tester pulled the tray back to its original position and lowered the Plexiglas screen. If the monkey instead responded to the incorrect window, the tester immediately lowered the Plexiglas screen. Response latencies on each trial were also recorded to the nearest 0.1 sec. These latencies were defined as intervals between the raising of the Plexiglas screen and the monkey's response. Each trial began 25 sec after the monkey' response on the preceding trial.

Experimental Design

All monkeys received 42 trials during each test day. The first two trials were warmup trials with a 0 sec delay between the offset of the window light and raising the Plexiglas screen. Responses on these trials were not used in the data analysis. The 40 subsequent trials were equally divided into five different delay conditions. In the constant (C) condition, the window light did not terminate after 2 sec, but remained on after the Plexiglas screen was raised until the subject responded to the window. Thus, the constant condition was equivalent to a simple brightness discrimination. Trials in the remaining four conditions were conventional delayed response in which the delay between the offset of the 2 sec cue light and the raising of the Plexiglas screen was 0, 6, 12, or 18 sec. Presentation order of the trials under each of the five conditions was randomized with the restriction that no condition be presented on two consecutive trials. The sequence of correct positions was randomized each day with the following

restrictions: a. No position was correct on more than three successive trials, b. Each delay condition was presented four times with right correct and four times with left correct, and c. For each condition, right correct and left correct trials were each preceded an equal number of times by right correct and left correct. As in Experiment 1, this balancing was nesessary to conduct the SST analysis of response sequences.

The sequence of drug conditions was randomized with the restriction that each of the three conditions be presented once during successive three-day blocks. In addition, no drug condition was ever presented on two successive days.

Response Sequence Analysis

The SST analysis applied to the data was a simplified version or the analysis used in Experiment 1. The delayed response problem contains fewer sources of systematic or nonrandom errors than does the object discrimination problems previously used. States whose strengths were measured in Experiment 2 included detect (D), positional win-stay;lose-shift (F), position perseveration (P), position preference (B), and random (R). State stregnths were defined the same as for the Experiment 1 analysis.

Results

Figure 2 displays the proportion of correct responses and the corresponding D values as a function of delay condition and level of DAME administered. The monkeys' accuracy fell monotonically with increasing delays [$F(2,20) = 26.28$, $p < .0001$]. The drug effect did not approach statistical significance. However, the drug by delay interaction was significant ($F(8,40) = 2.26$, $p = .0425$), clearly as a result of the divergence of performances in the three drug conditions at the longest delay (18 sec). At this delay, performance increased in a dose related manner; at other delays, performance in the three drug conditions were comparable. Analysis of 18 sec delay data showed a significant overall difference among the three drug conditions [$F(2,10) = 13.40$, $p = .0015$]. Individual comparisons showed that fewer errors occurred in the 500 microgram condition than in the 100 microgram condition [$F(1,10) = 8.71$, $p < .05$] and in the saline control condition [$F(1,10 = 26.71$, $p < .001$]. The latter two conditions did not differ significantly.

Figure 3 shows the corresponding data for the bias free measure K. The K values closely paralleled those for proportion of correct responses. Significant delay [$F(4,20) = 8.28$, $p = .0004$] and drug by delay [$F(8,40) = 3.05$, $p = .0090$] effects occurred, but the drug effect was not significant. Again, the interaction was attributable to a significant drug effect at the 18 sec delay [$F(2,10) = 13.26$, $p = .0014$]. Analysis of individual comparisons showed that significantly more errors

occurred in the saline control condition than in the 100 microgram [$F(1,10) = 13.57$, $p < .01$] and in the 500 microgram conditions [$F(1,10) = 25.43$, $p < .001$]. Performance did not differ significantly between the two different levels of DAME.

The mean strength of positional win-stay;lose-shift (state F) was virtually zero (mean = -0.0067). Three monkeys had small positive means for this state and three had small negative means. No significant effects involving state F occurred.

Position perseveration (state P) had a mean value of 0.07, a value that although small nevertheless significantly exceeded zero [$t(5) = 6.19$, $p < .01$]. Analysis of variance, however, showed no significant variation of state P involving either the drug or the delay variable. Position preference (state B) had a mean value of 0.09 and increased significantly with delays [$F(4,20) = 4.47$, $p = .0096$]. No other effects were significant.

Since positional win-shift;lose-stay was virtually zero, its value was not included in the subtractive process defining random responding (state R). Therefore, the modified R was defined as 1 - P - B. The mean strength of R was 0.12. This value significantly exceeded zero [$t(5) = 3.43$, $p < .02$]. Random responding increased significantly with delay [$F(4,20) = 5.51$, $p = .0037$], but no effects involving the DAME variable were significant.

The proportion of total errors accounted for by position perseveration, position preference, and random responding were also calculated. These proportions were defined by the ratio of the state strength to 1 - D. Thus, for example, the proportion of errors attributable to position perseversation was P / (1 - D). None of the three resulting proportions varied significantly with delay or DAME levels.

Figure 4 shows response latencies as a function of delay condition and DAME level. Latencies were about 0.1 sec longer in the saline control condition than in the two DAME conditions [$F(2,10) = 4.26$, $p = .0460$] which yielded virtually identical latencies. Separate analyses showed that the DAME related decrease in response latencies was present in the four delayed response conditions (0, 6, 12, and 18 sec) [$F(2,10) = 5.19$, $p = .0285$] but not in the constant condition. Latencies also varied slightly but significantly over the five delay conditions [$F(4,20) = 3.30$, $p = .0313$]. Latencies increased significantly from 0 to 18 sec [$F(3,15) = 3.88$, $p = .0309$]. In addition, latencies were about 0.1 sec less in the 0 sec than in the constant condition [$t(5) = 2.76$, $p < .05$].

Discussion

Experiment 2 provided substantial evidence that DAME in dosages from 100 to 500 micrograms/kg increases memory for two-choice spatial delayed response in squirrel monkeys. Two

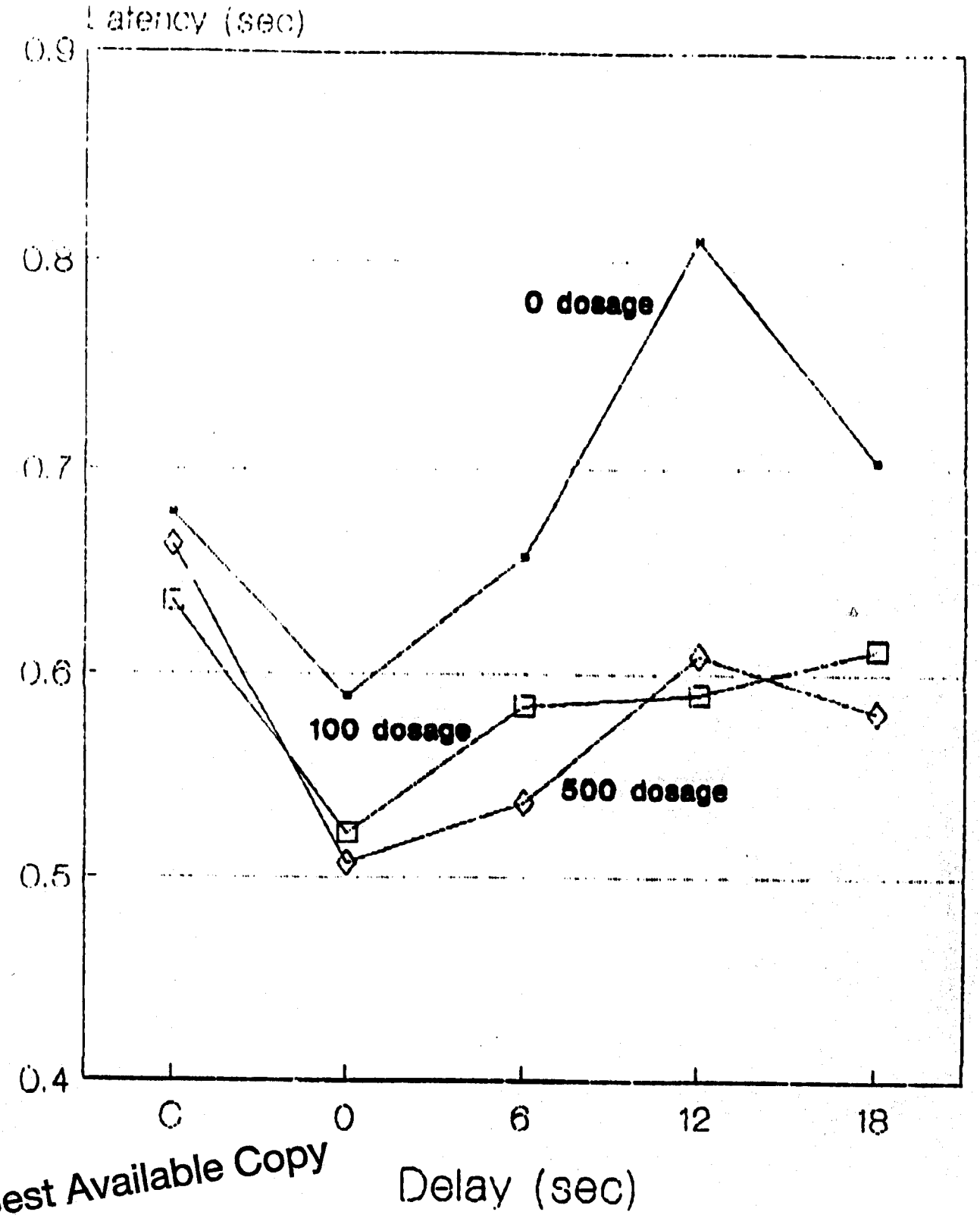

Fig. 4 Response latencies as a function of delay conditions and DAME dosage. Dosage values refer to micrograms/kg. C refers to constant condition.

basic findings support this contention. The first was the delay by drug level interaction for the proportion of correct responses (or state D). DAME facilitated performance after 18 sec delays but not after shorter delays. This result indicates that DAME did not occasion a generalized and nonspecific reduction of errors independent of delay, but instead only facilitated performance when the memory demand approach about 18 sec. A retention interval by condition interaction, in which the conditions effect increases with retention interval is, a conventional criterion for a memory effect (see Bartus, 1979). The second finding that indicated DAME's effect on memory was the invariance of the drug by delay interaction when the bias free measure K was the dependent variable. This interaction showed that the effect of DAME was not an artifact resulting from suppression of stereotyped responding at the longer delays. For example, assume that the monkeys became increasingly distracted or excited as delay increased and consequently displayed corresponding increases in position habits or some other systematic error. If DAME had simply reduced the distractibility of the monkeys, then a drug dosage by delay interval interaction would have been expected for proportion of correct responses but not for the bias free K. Since the interaction in terms of K was just as clear as that in terms of proportion of correct responses, it follows that DAME did not simply affect systematic error production. The absence of any significant DAME effect on any of the systematic errors is also consistent with this interpretation.

The parallel effects of DAME on number of correct responses and on the bias free measure K also occurred in Experiment 1 which included a different monkey species as well as a different problem. Yet, in Experiment 1, DAME impaired multiple discrimination reversal performance while in Experiment 2 it facilitated memory. Apparently DAME does not affect emission of systematic errors in two choice problems nor is its effect mediated through an effect on frequency of systematic errors.

As noted before, previous research on met- and leu-enkephalin analogues in appetitively motivated memory has shown effects ranging from no effect to impairment (Olson et al., 1979; Olson et al., 1981; Linden & Martinez, in press). More generally, opiate peptides have typically impaired memory, including aversively motivated problems, and opiate antagonists have improved it (Olson, Olson, & Kastin, 1986). Results from the present experiment make the inconsistency of the reported effects complete by apparently being the first to demonstrate improved memory resulting from enkephalin administration in an appetitively motivated problem. The confusing mixture of results has no readily apparent explanation and further underscores the error of assuming that memory is a single process measurable in any sort of problem requiring delayed expression of earlier learning. Indirect delayed response in squirrel monkeys, direct delayed response in rhesus monkeys, retention of maze habits in rats, and retention of passive

avoidance by rats are vastly different problems. Nevertheless, all are frequently described as tests of memory. Species differences as well as differences in the characteristics of the problem learned are obvious sources of discrepant results with the same types of enkephalins. Experiments 1 and 2 suggest another class of variables that may be a source of discrepancy, namely the strength of systematic relative to nonsystematic errors. Systematic errors accounted for a small portion of all responses in both experiments, possibly because of the extensive previous experience of the subjects. In problems engendering a high level of systematic errors, the effects of enkephalin may be dramatically different.

Response latencies increased with delays varying from 0 to 18 sec. In all delay conditions, raising the transparent Plexiglas screen at the end of the delay was a cue indicating that a response could be made. Therefore, increased latencies must be attributed to longer times from window light offsets to response availability during longer delays. This indicates that the window lights had an excitatory effect on readiness to respond that diminished over time. The increased latencies were themselves negligible contributors to the decreased response accuracy at the longer delays since the total increase in latency from 0 to 18 sec was only about 0.1 sec. Parallel decreases in response accuracy and response speed seen in Figures 3 and 4 suggest that a common memory related process underlies both processes. A direct association between an excitatory measure, such as response latency, and a measure of choice accuracy, such as proportion of correct responses or the bias free measure K, is not without implications for theoretical treatments of delayed response and delayed matching.

DAME administration also resulted in reduction of response latencies by about 0.1 sec. However, DAME produced similar reductions in latency at all delays whereas it enhanced response accuracy only during the 18 sec delay trials. Therefore, these two DAME related phenomena probably involve different processes.

EXPERIMENT 3

The delayed response problem used in Experiment 2 was a discrimination between two fixed locations. The monkeys' performance was probably substantially based upon retention of either overt or covert bodily orientation towards the positive location. This type of spatial problem has a variety of names including taxon system (O'Keefe & Nadel, 1978) and personal or egocentric orientation (Semmes, Weinstein, Ghent, & Teuber, 1963). The two-choice problem is clearly different from a spatial memory problem whose solution requires remembering a location independently of personal orientation. A variation of delayed response not as completely bound to bodily orientation as the two-choice problem is the response matrix version which has a greater component of purely spatial memory (see Bartus, Fleming, & Johnson, 1978; Medin, 1969). In the response matrix problem, response locations are located within either three by three or four by four matrices with 9 or 16 possible choices, respectively.

Nine-choice response matrix problems were used in Experiment 3. In Experiment 2, DAME enhancement of performance occurred with delays of 18 sec but not for delays of 12 sec or less. A question posed by this result is whether the enhancement only occurs after relatively long delays (viz. 18 sec or more) or whether it would also occur after shorter delays when the problem is made more difficult by increasing the number of choices beyond two. In addition, since performance on the nine-choice problem could not be as easily mediated by simple bodily orientation to the correct location as could performance on the two-choice problem. Experiment 3 provided information about the generality of the DAME produced error reduction when different memory storage strategies are used.

Method

Subjects

Subjects were the same four male and two female squirrel monkeys that served in Experiment 2.

Apparatus

The monkeys were tested in the same transport/test cages used in Experiments 1 and 2; however, the response panel contained nine recessed windows arranged in a three by three matrix. Each window was a square, 5 1/2 cm on a side, constructed from perforated plastic with conductive copper coating. The distance between adjacent edges of the windows was 5 cm. Illumination from incandescent lights behind each window produced a distinctive visual display through the perforations. A small wooden drawer containing a foodwell was located immediately below each window. As in Experiment 2, correct responses were rewarded by pushing the drawer under the chosen

window toward the monkey, withdrawing the drawer after the monkey had retrieved the reward. A transparent Plexiglas screen, located between the monkey and the response panel, was raised and lowered manually by the tester.

Monkeys registered responses by touching one of the nine windows on the response panel, completing a high-impedance, 5 volt circuit with 0.25 microamps maximum current flow. An attached Apple IIe computer controlled the presentation of window lights, signaled the tester when to raise and lower the Plexiglas screen, presented a 1 sec tone following correct responses, and recorded the position and latency of each response.

Procedure

The pretest injection procedure was identical to that used in Experiment 2. Monkeys were administered subcutaneous injections of physiological saline or DAME at dosages of either 100 or 500 micrograms/kgm 15 minutes before the start of testing. Solutions were acidified with acetic acid to a ph of approximately 4.2.

The testing procedure was also virtually identical to the Experiment 2 procedure. Trials were initiated by 2 sec illumination of one window light while the transparent Plexiglas screen was down. At the end of the delay period, the tester raised the screen giving the monkey access to the response panel. If the monkey touched the previously lighted window, the 1 sec tone sounded and the monkey was rewarded. Response latencies were recorded by the computer to the nearest 0.1 sec. As before, latencies were intervals between raising of the Plexiglas screen and the monkeys' responses. The intertrial interval was 25 sec.

Experimental Design

Each test day, 54 trials were presented. Delays between window light offsets and the raising of the Plexiglas screen were 0, 4, or 8 sec. Each delay interval was presented on 18 trials each day with the following three restrictions that were generalizations of the restrictions used in Experiment 2. First, the same delay was never presented on two consecutive trials. Second, each of the nine windows was correct equally often for each of the delays. Third, for each delay, on 16 of the 18 daily trials the correct window was different from the correct window on the preceding trial. On the other two trials (1/9 of the total) the correct window was the same as on the preceding trial. The presentation of the three different DAME injection conditions was randomized according to the same schedule used in Experiment 1.

Response Sequence Analysis

Since Experiment 3 incorporated nine-choice trials instead of the two-choice trials used in Experiments 1 and 2, the SST analysis had to be based on generalizations of the formulas used previously. As in Experiment 2, values were obtained for states D (detect), F (positional win-stay;lose-shift), P (position perseveration), B (position preference) and R (random responding). Derivations of the generalized formulas are given below.

a. In the nine-choice problem, the probability of a correct response in any nondetect state is 1/9. Therefore, $Po(+_2) = D + 1/9(1 - D)$ and $D = [9Po(+_2) - 1] / 8$.

b. In the two-choice problem, the observed probability of a win-stay;lose-shift response, Po(w-s;l-s), is $F + 1/2(1 - F)$. In the nine-choice problem, the probability of a win-stay;lose-shift response in a non F state also has two components, one for win-stay, the other for lose shift. The probability of a win-stay response in a non F state is 1/9 and the corresponding probability of a lose-shift response is 8/9. Both of these components are weighted equally and independently of the number of wins or loses. Therefore, the win-stay;lose-shift proportion under all non F states is simply the mean of 1/9 and 8/9 or 1/2. Thus, for the nine-choice problem, $Po(w\text{-}s;l\text{-}s) = F + 1/2(1 - F)$ and $F = Po(st_2 | +_1) + Po(sh_2 | -_1) - 1$ as in the two-choice problem.

c. In the nine-choice problem, the observed proportion of perseverative responses, $Po(p) = P + 1/9(1 - P)$ from which it follows that $P = [9Po(p) - 1] / 8$. Nine different types of perseverative responses exist, one for each of the nine response alternatives. The estimate of Po(p) is the unweighted mean of the proportions of all nine possible perseverative responses. If C_{i1} and C_{i2} represent choices of window i (i = 1,2,3,...9) on trials 1 and 2 respectively, then $Po(p) = [\sum_{i=1}^{9} Po(C_{i2} | C_{i1})] / 9$.

d. The rationale for calculating a value for position preference, B, in the nine-choice problem is to assume that from 1 to 8 windows will elicit preferences such that the observed proportion of choices to those windows will exceed 1/9 when all windows are rewarded equally often. Assume that n of the nine choices elicit observed response probabilities greater than 1/9. In this case, separate bias values will exist for each of the n windows, namely $B_1, B_2, B_3, \ldots B_n$. Since the probability of choosing window 1 in any non bias state is 1/9, it follows that the probability of choosing window 1, $Po(C_1)$ is $B_1 + 1/9(1 - B_1 - B_2 - B_3 - \ldots B_n)$. Unless only one window elicits a choice probability greater than 1/9, this equation will have more than one unknown and will consequently lack a unique solution. However, a separate equation can be defined for each window that attracts an above chance response frequency. Assume that, for example, three windows are chosen with above chance frequencies, viz. C_1, C_2, and C_3. Then,

$Po(C_1) = B_1 + 1/9(1 - B_1 - B_2 - B_3)$,
$Po(C_2) = B_2 + 1/9(1 - B_1 - B_2 - B_3)$, and
$Po(C_3) = B_3 + 1/9(1 - B_1 - B_2 - B_3)$.

After simplification,

$8B_1 - B_2 - B_3 = 9Po(C_1) - 1,$

$-B_1 + 8B_2 - B_3 = 9Po(C_2) - 1,$ and

$-B_1 - B_2 + 8B_3 = 9Po(C_3) - 1.$

These equations can then be easily solved for B_1, B_2, and B_3. Generalization to contexts with any number of unknown B values is obvious; B is then the sum of the component B values. Thus, $B = \sum_{i=1}^{n} B_i$, where n windows are chosen with above chance frequency.

e. Calculations of R and K are the same as in the two-choice problem. Consequently, R = 1 - D - F - P - B and K = D / (D + R).

Results

The percentage of correct responses and D decreased with increasing delays [F(2,10) = 18.60, p = .0004]. These measures also varied significantly with DAME level [F(2,10) = 5.37, p = .0260] in a curvilinear pattern. Performance fell as dosage increased to 100 micrograms/kg, but then rose to control levels as dosage increased further to 500 micrograms/kg. The curvilinear change resulted in a significant quadratic component to the DAME effect [F(1,5) = 9.31, p = .0284].

Variation in the bias free measure K, with delay and DAME levels, was parallel to that for percentage of correct responses. Performance declined with delay [F(2,10) = 10.63, p = .0033] and varied with DAME levels [F(2,10) = 4.29, p = .0450]. Figure 5 shows the effects of DAME level and delay on D and on K.

As in Experiment 2, values for win-stay;lose-shift (state F) were negligible with three monkeys having mean values that were slightly positive and three monkeys having mean values that were slightly negative. The overall mean value of F was -0.0197.

Position perseveration (State P) increased over delays [F(2,10) = 8.43, p = .0072] although the values were small. The increase with delay is depicted in Figure 6. The mean value of state P was 0.024 [t(5) = 4.07, p < .01]. Position perseveration did not vary with DAME levels.

Position preference (state B) increased significantly over delays [F(2,10) = 15.79, p = .0008] but was not significantly affected by DAME levels. Figure 6 displays position preference as a function of delays.

Since the strength of state F was virtually zero, values for random responding (state R) were calculated by subtracting values of D, P, and B, but not F from 1. This procedure was also followed for the analysis of Experiment 2 for the same reason. As shown in Figure 6, random responding increased linearly over delays [F(2,10) = 8.43, p = .0072]. DAME levels

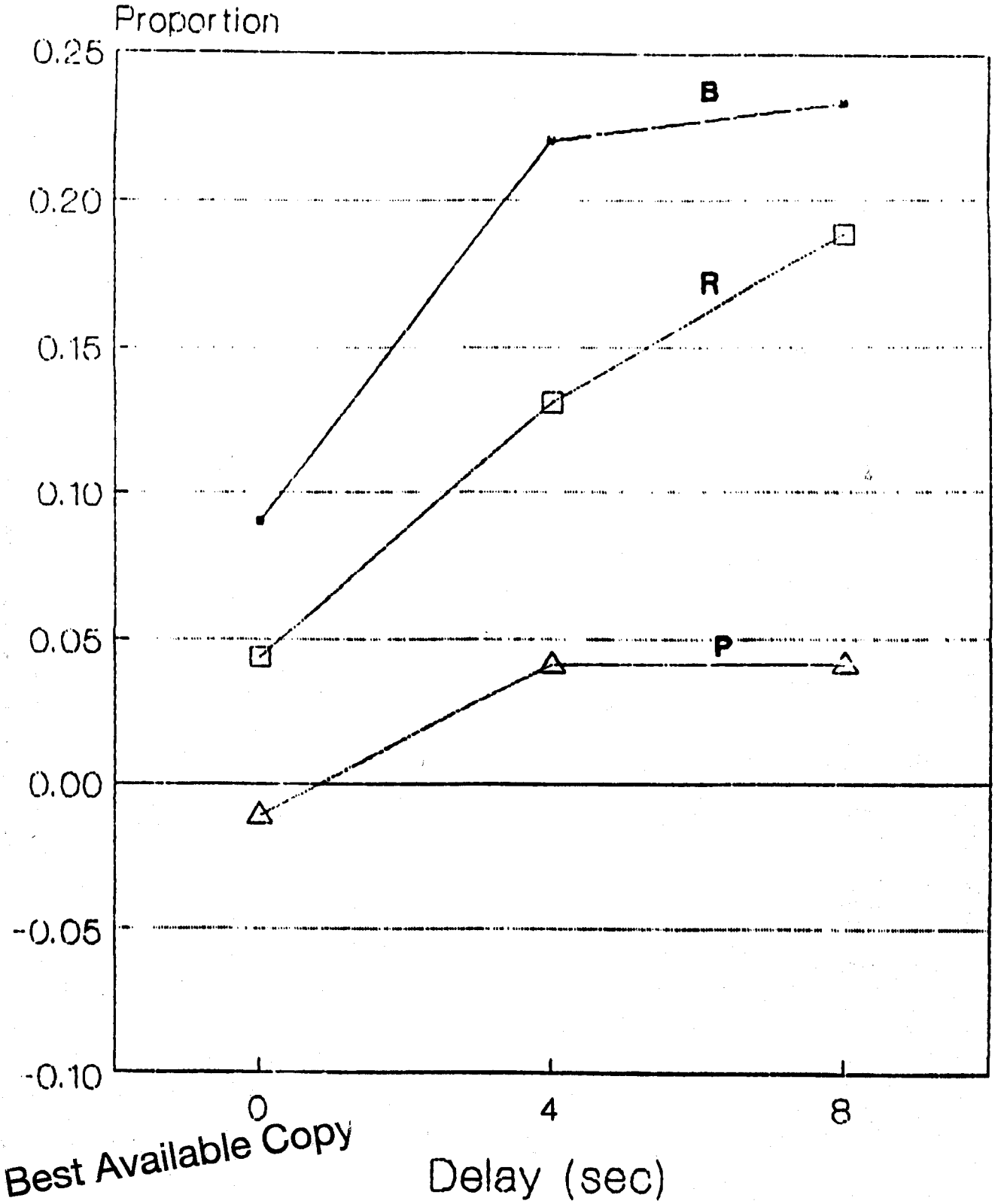

Fig. 6 Proportion of responses attributable to position perseveration (P), position preference (B), and random responding (R) as a function of delay.

did not exert a significant effect on random responding.

The proportion of total errors accounted for by position preference was calculated as in Experiment 2 by forming the ratio B / (1 - D). This ratio decreased significantly with delays [$F(2,10) = 7.68$, $p = .0095$]. Thus, although position preferences increased with delays concomitantly with position perseveration and random responding, the proportion of resultant errors attributable to position preference actually decreased from .72 to .63 to .52 as delay increased from 0 to 4 to 8 sec.

Response latencies were not affected significantly by dosages of DAME. However, they did increase slightly with delay [$F(2,10) = 4.40$, $p = .0426$]. Mean response latencies for 0, 4, and 8 sec delays were 0.88, 1.04, and 1.12 sec.

Discussion

The most striking outcome of Experiment 3 was the lack of a DAME related memory enhancement of the type that occurred in Experiment 2 with two-choice delayed response problems. Instead, overall performance declined from control levels with 100 microgram/kg doses but then increased to approximately control levels with higher 500 microgram/kg doses. The reduction in performance was mainifested in the percentage of correct responses as well as in the bias free measure K. Thus, as in Experiment 2, DAME effects were attributable to the peptide's effect on processes that neither stimulated nor suppressed systematic errors.

The different effects of DAME injections in Experiments 2 and 3 cannot be attributed to different levels of overall performance in the two experiments. The mean percentage of correct responses on the two-choice problems presented in Experiment 2, after 18 sec delays, was 74% (D = .48). The corresponding percentage on the nine choice problems presented in Experiment 3, after 8 sec delays, was 59 % (D = .54). The comparability of the D values shows that performance in these two conditions was similar after correction for the greater chance probability of a correct response in the nine-choice context. Yet, these were the two delay conditions at which DAME caused opposite effects in the two experiments. The impairment for low DAME doses in Experiment 3 is consistent with the previously noted tendency of opioids to retard rodent memory in aversively motivated tasks (Olson et al., 1981).

The different effects of DAME in the two experiments may be attributable to different memory strategies elicited by the two types of stimulus displays used. The nine-choice display was not as conducive to maintenance of bodily orientation towards the positive window as was the two-choice display. Maintenance or reestablishment of a gross orientation towards either the right or the left side after the delay was all that was necessary for storage of predelay information in the two-choice

problem. However, use of this strategy in the nine-choice problem would require maintenance of a far more precise and well controlled orientation to one of nine windows in a tightly packed array. Since squirrel monkeys are highly active and distractible (Fragaszy, 1985), they probably did not use this maintained orientation strategy during the nine-choice problems. Therefore, a more covert memory strategy may have been required to solve the nine-choice problems, a strategy that was disrupted by low doses of DAME.

Another difference between problems in Experiments 2 and 3 was the extent to which they elicited position preference errors. Position preference was the strongest source of systematic errors in both experiments. The increase in position preference errors with number of choices can be demonstrated by comparing strengths of position preference and random responding after 18 sec delays on the two-choice problems and after 8 sec delays on the nine-choice problems. Correct performance as measured by D was similar in both conditions. After 18 sec delays in two-choice problems, 13.2 % of the responses were attributable to position preference and 27.0% to random responding. However, after 8 sec delays in the nine-choice problem, 23.9% of the responses were attributable to position preference and 18.8% to random responding. The capacity of DAME to reduce correct performance may be strongest in problems that engender high levels of systematic error production.

Respose latencies increased by about 0.2 sec as delays increased from 0 to 4 sec, a result similar to that observed in Experiment 2. Response latencies were not significantly affected by DAME. As in Experiment 2, the slight increase in latencies with increasing delays probably had only a negligible effect on performance. The lack of any DAME effect on latencies in Experiment 3, as well as in Experiment 1, is evidence that when DAME does retard correct performance, it does not retard response latencies. Thus, explanations of DAME's impairment of correct performance in terms of its presumed effects on attention, vigilance, or activity levels are not supported by the data.

CONCLUSIONS

1. DAME does not have consistent effects upon appetitively motivated discrimination learning and delayed response of monkeys. The peptide impairs performance of capuchin monkeys on multiple discrimination reversal problems. Low (100 micrograms/kg) but not high (500 micrograms/kg) doses of DAME likewise impair nine-choice delayed response performance of squirrel monkeys. However, two-choice delayed response performance at long delays was enhanced by DAME.

2. Effects of DAME on primate learning and memory tasks is not attributable to interaction of DAME with the monkeys' responses to irrelevant or distracting stimuli.

3. Learning and memory functions plotted in terms of a bias free measure (K) parallel those plotted in terms of correct responses. DAME effects are therefore not attributable to strenghtening or reducing the relative number of systematic errors.

4. Response latencies were not affected by DAME dosages in experiments that demonstrated a DAME related reduction in performance. This result indicates that the performance reductions were not simply artifacts produced by drug related changes in activity level, or attention to the discriminative stimuli.

5. Results of these experiments provide no support for the use of DAME or related met-enkephalin analogs as general agents for enhanced performance on decision making or vigilance tasks.

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